

**Cyclohexane, Oxidized, Aq. Ext.**

**CAS Number 68915-38-8**

**201-15012A**

**A Mixture Also Known as:**

- ☐ EP-306 Acid Water
- ☐ COP Acid Water
- ☐ Dicarboxylic Acid Solution
- ☐ Dicarbonsaeure Loesung A Ber. Trocken
- ☐ Dicarbonsaeure Loesung L Ber. Trocken
- ☐ Abstreifsaeure

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**U.S. EPA HPV Challenge Program Submission**

**December 30, 2003**

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## Executive Summary

EP-306 is an aqueous mixture of monocarboxylic acids (C1 – C6), dicarboxylic acids (C4-C6), 6-hydroxycaproic acid, esters of alcoholic and acidic components, and trace amounts of oxidized cyclohexane, which is produced as a site-limited intermediate in the purification of cyclohexanone. During the purification process, the oxidized cyclohexane stream is washed with water to remove the soluble components described above. The water wash is then concentrated to form EP-306. EP-306 is stored on-site and, due to its high content of adipic acid and 6-hydroxycaproic acid, used as a feed stock for the on-site manufacture of 1,6-hexanediol. Any material not used is disposed of on-site via deep well injection. Annual production ranges between 50 and 75 million pounds. Due to its use only on-site, potential human exposures are limited to site personnel who may contact the product during manufacturing upsets and routine diagnostic checks.

The physical-chemical properties of EP-306 encompass those of its components. At 20 °C, EP-306 is a liquid / solid slurry with a low vapor pressure. Its components are expected to partition to water in the environment, where they are both soluble and stable with little potential for bioaccumulation due to their relatively low log  $K_{ow}$  values (-0.6 to 2). EP-306 is biodegradable in a wastewater treatment facility. The photochemical half-lives of the major EP-306 components, adipic acid and 6-hydroxycaproic acid, in air are 1 – 2 days, although several minor components have half-lives of about 20 days. Fish, *daphnids*, and algae are acutely affected by EP-306 with  $LC_{50}$  /  $EC_{50}$  values of 220 – 460 mg/l, > 100 mg/l and 27 -35 mg/l, respectively. Data in fish indicate that these effects may be due to changes in pH, rather than the intrinsic toxicity of EP-306 components themselves.

EP-306 or major components exhibit low acute toxicity by all three routes of potential exposure with  $LD_{50}$  /  $LC_{50}$  values of: > 5000 mg/kg (oral, EP-306), > 7940 mg/kg (dermal, adipic acid), and > 7.7 mg/l (inhalation, adipic acid). *In vitro* data for EP-306 and *in vivo* data on major components indicate that EP-306 is neither mutagenic nor clastogenic. Chronic toxicity data are not available for EP-306 itself. However, data sufficient to meet these needs are available for adipic acid, the major component of EP-306. Adipic acid data can also be used as a surrogate for 6-hydroxycaproic acid, the second most prevalent component in EP-306, as 6-hydroxycaproic acid will be metabolically converted to adipic acid once absorbed into the body. As these two chemicals are the most prevalent components in EP-306, it is likely that they also constitute a major fraction of the unidentified esters in EP-306. In the body, the EP-306 esters would be hydrolyzed by carboxyesterases to their alcoholic and acidic components. Thus, the total fraction of these two chemicals in EP-306 constitutes approximately 90 % of the organic components in EP-306. In a two-year rat feeding study, adipic acid exhibited a NOAEL of ~ 500 mg/kg; 4-fold greater doses affected only food consumption and body weight gain. Male and female reproductive organs were not affected at any dose. In a developmental study, rats were gavaged with adipic acid. No effects were observed on maternal or fetal survival, reproductive outcomes, embryotoxicity, or teratogenicity at the highest dose tested (288 mg/kg). These data are sufficient to assess the repeated-dose, reproductive and developmental hazards posed by EP-306, particularly given its use as a site-limited intermediate.

Based on the available information, no further testing is recommended for EP-306.

## Testing Plan and Rationale

CAS # 68915-38-8		Information Available?						
EP-306		OECD Study?						
HPV Endpoint		GLP Study?						
Physical Chemical		Supporting Information?						
Melting Point	Y	N	N	N	N	Y	N	
Boiling Point	Y	N	N	N	N	Y	N	
Vapor Pressure	Y	N	N	Y	Y	Y	N	
Partition Coefficient	Y	N	N	Y	Y	Y	N	
Water Solubility	Y	N	N	Y	Y	Y	N	
Environmental & Fate		Estimation Method?						
Photo-Degradation	Y	N	N	Y	Y	Y	N	
Water Stability	Y	N	N	Y	N	Y	N	
Transport	Y	N	N	Y	Y	Y	N	
Biodegradation	Y	Y	Y	Y	Y	Y	N	
Ecotoxicity		Acceptable?						
96-Hour Fish	Y	N	N	Y	N	Y	N	
48-Hour Invertebrate	Y	Y	Y	Y	N	Y	N	
96-Hour Algae	Y	N	N	Y	N	Y	N	
Toxicity		Testing Recommended?						
Acute	Y	N	N	Y	N	Y	N	
Genetic Toxicology <i>in vitro</i>	Y	Y	Y	Y	N	Y	N	
Genetic Toxicology <i>in vivo</i>	Y	N	N	Y	N	Y	N	
Repeated Dose	Y	N	N	Y	N	Y	N	
Reproductive	Y	N	N	Y	N	Y	N	
Developmental	Y	N	N	Y	N	Y	N	

## 1.0 Introduction

The aqueous extract of oxidized cyclohexane (designated EP-306 by BASF Corporation)(CAS No. 68915-38-8) is a yellow-colored intermediate produced during the production and purification of cyclohexanone. Cyclohexanone is produced via the cyclohexane oxidation route for the manufacture of caprolactam. By-products formed during this oxidation process must be removed as part of the overall purification of cyclohexanone. Purification is accomplished in part via a water wash to remove most of the soluble mono and dicarboxylic acids from the oxidized cyclohexane stream. The water wash is then concentrated to form EP-306. As indicated in Table 1, the primary components of EP-306 are water (~ 50 %), adipic acid (~ 18 %) and 6-hydroxy caproic acid (~ 12 %). EP-306 is transported via pipeline to large on-site storage tanks. From here, it is transported via pipeline as a raw material feed stock for the on-site manufacture of 1,6-hexanediol. The two primary organic components are separated from EP-306 during this process. Seventy percent of EP-306 is used for the manufacture of 1,6-hexanediol; the remainder is sent to an on-site deep well facility for disposal. The annual production of EP-306 ranges between 50 and 75 million pounds. Since EP-306 is an intermediate that is made, stored and consumed on-site, potential human exposures are limited to operational personnel who may contact EP-306 during either manufacturing upsets or system sampling for quality and routine diagnostic checks.

**Table 1. Typical Composition of EP-306**

<b>EP-306 Component</b>	<b>Weight %</b>
Formic Acid	1 – 3
Acetic Acid	0.05 - 0.3
Propionic Acid	0.05 – 0.3
Butyric Acid	0.2 – 0.6
Valeric Acid	0.2 – 0.6
Caproic Acid	0.05 - 0.1
Succinic Acid	0.3 – 1
Glutaric Acid	1 – 2
<b>Adipic Acid</b>	<b>12 – 24</b>
<b>6-Hydroxycaproic Acid</b>	<b>10 – 14</b>
Cyclohexanol	0.1 – 0.4
Cyclohexanone	0.05 – 0.2
Cyclohexyl Hydroperoxide	0.1 – 0.2
Other*	5 – 10
<b>Water</b>	<b>40 – 60</b>

\* Primarily esters of 6-hydroxycaproic acid and acidic components

Information and study results on EP-306 as well as surrogate chemicals (adipic acid and a dicarboxylic acid mixture) are briefly reviewed in this rationale document to indicate how they meet the Screening Information Data Set (SIDS) endpoints of the United States Environmental Protection Agency (USEPA) High Production Volume (HPV) Challenge program. Robust summaries of studies on EP-306 are attached to this document; robust summaries and/or information for EP-306 surrogates are referenced at the end of this document.

## 2.0 Physical-chemical Data

Physical-chemical data for EP-306 are available from the manufacturer and are summarized in Table 2.

**Table 2. Physical-chemical Properties of EP-306**

Melting Point	-6 to 45 °C (1,2)
Boiling Point	101 °C @ 1013 hPa (2)*
Vapor Pressure	77 hPa @ 40 °C (2)*
Partition Coefficient	Log K <sub>o/w</sub> = -0.59 to 1.92 (3,4)
Water Solubility	Miscible (2, 4)

\* Initial Value

The data indicate that EP-306 is a liquid/solid mixture at room temperature. The mixture begins to boil at a temperature slightly higher than that of water. The upper end of the boiling point range is anticipated to be ~ 338 °C, the boiling point for adipic acid, the known EP-306 component with the highest boiling point (3). The initial vapor pressure for EP-306 is slightly higher than that of water, likely due to the presence of formic acid, the only EP-306 component with a vapor pressure greater than that of water (3). As the mixture evaporates and loses the more volatile components, the vapor pressure will decrease and eventually approach  $\sim 4 \times 10^{-7}$  hPa, the vapor pressure of adipic acid, the least volatile known component of EP-306 (3).

**Table 3. Log K<sub>o/w</sub> and Water Solubility of Known EP-306 Components**

EP-306 Component	Log K <sub>o/w</sub> (4,5)	Water Solubility (5) (mg/l)
Formic Acid	-0.54 (exp)	955,000 (cal)
Acetic Acid	-0.17 (exp)	476,000 (cal)
Propionic Acid	0.33 (exp)	174,000 (cal)
Butyric Acid	0.79 (exp)	66,100 (cal)
Valeric Acid	1.39 (exp)	18,600 (cal)
Caproic Acid	1.92 (exp)	5,900 (cal)
Succinic Acid	-0.59 (exp)	808,000 (cal)
Glutaric Acid	-0.29 (exp)	396,000 (cal)
<b>Adipic Acid</b>	<b>0.08 (exp)</b>	<b>167,000 (cal)</b>
<b>6-Hydroxycaproic Acid</b>	<b>0.59 (cal)</b>	<b>229,000 (cal)</b>
Cyclohexanol	1.23 (exp)	33,700 (cal)
Cyclohexanone	0.81 (exp)	24,100 (cal)
Cyclohexyl Hydroperoxide	1.85 (cal)	2,750 (cal)

(exp), experimental value; (cal), calculated value

A single octanol-water partition coefficient is inappropriate for a mixture with components of varying hydrophilicities. To understand the potential distribution and bioaccumulative properties of EP-306, individual components must be taken into consideration. Table 3 contains the experimental and calculated log Ko/w values listed in EPI Suite (4,5). The range of Log Ko/w values suggests that EP-306 has little potential for bioaccumulation. All EP-306 components are soluble in water, with solubilities at 25 °C ranging from 2,700 mg/l (cyclohexyl hydroperoxide) to 955,000 mg/l (formic acid)(5).

**Recommendation:** No additional physical-chemical studies are recommended. The available data fill the HPV required data elements with sufficient precision to define potential hazards of this mixture.

### 3.0 Environmental Fate and Pathways

Photodegradation was estimated using version 1.90 of the Atmospheric Oxidation Program (AOP) Program that estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals (6). The estimated rate constant is used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radical. The approach used was to take the components of EP-306 and individually determine their reactivity with hydroxyl radical assuming each component will be unaffected by the others after vaporization into the troposphere. Using the default atmospheric hydroxyl radical concentration in AOP program and the range of estimated rate constants of major components of EP-306 for reaction with hydroxyl radical, the estimated half-life of EP-306 components in air is approximately 1 to 20 days. Table 4 below provides a summary of the results.

**Table 4. Half-Life of Known EP-306 Components**

<b>EP-306 Component</b>	<b>Half-Life (6) (Days)</b>
Formic Acid	20.6
Acetic Acid	17.2
Propionic Acid	7.71
Butyric Acid	3.96
Valeric Acid	2.60
Caproic Acid	1.94
Succinic Acid	3.87
Glutaric Acid	2.56
<b>Adipic Acid</b>	<b>1.91</b>
<b>6-Hydroxycaproic Acid</b>	<b>1.09</b>
Cyclohexanol	0.61
Cyclohexanone	0.88
Cyclohexyl Hydroperoxide	0.76

Theoretical Distribution (Fugacity) of EP-306 in the environment was estimated using the MacKay EQC level III model with standard defaults in EPI Suite (v 3.10) using 100% release to water as the means of entry into the environment (7). The approach used was to estimate the fugacity of each EP-306 component assuming that one component will not greatly affect the distribution of the other. It is apparent that the major components distribute almost exclusively to water. Although the hydrolytic stability of EP-306 in water has not been tested, carboxylic acids, alcohols and ketones are organic functional groups that are known to be generally resistant to hydrolysis and therefore are considered to have an estimated half-life in water of more than 1 year (8). The only component that may be lost to the hydrolytic process in water is cyclohexyl hydroperoxide. Although organic hydroperoxides are typically stable in pure water, they can be reduced to the alcohol in the presence of oxidizable substrates and metal ions.

The biodegradability of EP-306 was measured under GLP conditions in a carbon dioxide evolution test (OECD 301 B). Results indicated that EP-306 is biodegradable as 70 % to 80 % of the theoretical carbon dioxide production occurred after 28 days (9). These results are consistent with the ready biodegradability measured for adipic acid (10), the major component of EP-306. In addition, BIOWIN (v4.00) modeled results for each component of EP-306 indicates that all are rapidly degradable (11).

**Table 5. Theoretical Distribution (Fugacity) of EP-306 Components in the Environment**

EP-306 Component	Percent Distribution (7)			
	Air	Water	Soil	Sediment
Formic Acid	0.006	99.8	0.021	0.15
Acetic Acid	0.003	99.8	0.015	0.15
Propionic Acid	0.023	99.8	0.029	0.15
Butyric Acid	0.025	99.8	0.027	0.16
Valeric Acid	0.018	99.8	0.023	0.18
Caproic Acid	0.027	99.7	0.024	0.23
Succinic Acid	<0.001	99.9	<0.001	0.15
Glutaric Acid	<0.001	99.9	<0.001	0.15
<b>Adipic Acid</b>	<0.001	99.8	<0.001	0.15
<b>6-Hydroxycaproic Acid</b>	<0.001	99.8	<0.001	0.15
Cyclohexanol	0.076	99.7	0.016	0.19
Cyclohexanone	0.315	99.5	0.032	0.18
Cyclohexyl Hydroperoxide	0.248	99.5	0.023	0.26

**Recommendation:** No additional fate and pathway studies are recommended. The available data fill the HPV required data elements.



## 4.0 Ecotoxicity

### 4.1 Toxicity to Fish

Overall, the measured results with EP-306, adipic acid (the major component of EP-306), as well as a mixture of C4 to C6 dicarboxylic acids indicate that EP-306 has a low acute ecotoxicity (Table 6). At 96 hours, the LC<sub>0</sub> and the no observed effect concentration (NOEC) for EP-306 were 215 mg/l; the next two higher concentrations (464 mg/l and 1000 mg/l) caused 100% mortality within 48 hours and 4 hours, respectively (12). In a parallel portion of this study, no mortality was observed at 96 hours when the pH of the EP-306 test solution (1,000 mg/l) was adjusted to 7.3. This study closely followed the OECD 203 guideline with the exception of the recommended fish species. These results are supported by LC<sub>50</sub> studies with EP-306 surrogates: undiluted adipic acid in the fathead minnow (97 mg/l)(13) and a dilute dicarboxylic acid mixture in the Rainbow trout (240 mg/l)(14) and Bluegill sunfish (340 mg/l)(15).

**Table 6. Measured Aquatic Toxicity of EP-306 and Surrogate Chemicals**

Endpoint	EP-306	Adipic Acid	C4 to C6 Dicarboxylic Acid Mixture
Fish, 96-hr LC <sub>50</sub>	220-460 mg/l (12)	97 mg/l (13)	240 - 340 mg/l (14, 15)*
<i>Daphnia</i> , 48-hr EC <sub>50</sub>	> 100 mg/l (16)	85.7 mg/L (17)	> 1,000 mg/L (18)*
Algae, 96-hr EC <sub>50</sub>	No Data	26.6 mg/l (19)	35 mg/l (20)

\*Containing: glutaric acid (16.43%), succinic acid (4.77%), adipic acid (3.9%), nitric acid (3.9%), and water (71%)

### 4.2 Toxicity to Aquatic Invertebrates

Aquatic invertebrate toxicity was examined in studies with *Daphnia magna*. In a GLP study by BASF following the OECD 202 protocol, the 48-hr EC<sub>0</sub> and EC<sub>50</sub> were > 100 mg/l (16). As with fish, these results are consistent with 48-hr EC<sub>50</sub> values obtained with EP-306 surrogates: undiluted adipic acid (85.7 mg/l)(17) and a dilute dicarboxylic acid solution (> 1,000 mg/l)(18). In the BASF study, analytical measurements of the test solutions were performed at the start (0 hr) and end (48 hr) of the test by quantification the 6-hydroxycaproic acid peak. At 0 hr, the analytical concentrations closely approximated the nominal concentrations. However, at 48 hr, the analytical concentrations were about 20% to 45% higher than expected, perhaps reflecting hydrolytic cleavage of the unspecified esters in EP-306 to 6-hydroxycaproic acid and one of the component acids.

### 4.3 Toxicity to Aquatic Plants

The toxicity of EP-306 to aquatic plants has not been evaluated. Data, however, are available on EP-306 components and surrogates. Adipic acid (purity unspecified)(19) and a mixture of C4 to C6 dicarboxylic acids (purity unspecified) were tested for their ability to inhibit the growth of *Scenedesmus subspicatus*; the 96-hr EC<sub>50</sub> values were 26.6 mg/l (19) and 35 mg/l (20), respectively. Other support for the low level of toxicity comes from modeling. The EPI Suites ECOSAR model estimates the 96-hr EC<sub>50</sub> values for adipic acid and 6-hydroxycaproic acid to be > 26,000 mg/l and > 11,000 mg/l, respectively (21). The difference in EC<sub>50</sub> values between experimental and modeled data may be due in part to an effect of pH.

**Recommendation:** No additional ecotoxicity studies are recommended. The available information fills the HPV required data elements.

## 5.0 Acute Mammalian Toxicity

### 5.1 Oral Toxicity

The oral LD<sub>50</sub> of EP-306 was determined to be greater than 5,000 mg/kg in Wistar rats (22). Rats (five males and 5 females) were gavaged with one of four doses ranging from 681 mg/kg to 5,000 mg/kg. The NOEC was 681 mg/kg. Symptoms of exposure included: apathy, dyspnea, staggered and spastic gait, piloerection, and a poor general state. Three of 10 rats died at 5,000 mg/kg; animals dying on study exhibited general congestion, emaciation, empty and distended gastrointestinal tract, and mucosal inflammation of the glandular stomach. No pathologic findings were observed in the survivors. These results are supported by oral LD<sub>50</sub> studies of EP-306 components and surrogates: dosed as a 20% adipic acid solution in corn oil (5,050 mg/kg)(23) and a C4 to C6 dicarboxylic acid mixture (composition and purity not specified)(6,629 mg/kg)(24).

**Table 7. Acute Toxicity of EP-306 and Surrogate Chemicals**

Endpoint	EP-306	Adipic Acid	C4 to C6 Dicarboxylic Acid Mixture
Oral LD <sub>50</sub> (rat)	> 5,000 mg/kg (22)	5,050 mg/kg (23)	6,829 mg/kg (24)*
Dermal LD <sub>50</sub> (rabbit)	No Data	> 7,940 mg/kg (25)	> 7,940 mg/kg (26)
Inhalation LC <sub>50</sub> (rat)	No Data	> 7.7 mg/l (27)	No Data

\*Containing: glutaric acid (60%), succinic acid (25%), and adipic acid (15%)

### 5.2 Dermal Toxicity

Although no acute dermal studies with EP-306 have been performed, studies exist for component and surrogate chemicals. The dermal LD<sub>50</sub> of adipic acid and a C4 to C6 dicarboxylic acid mixture in rabbits were both determined to be greater than 7,940 mg/kg (25, 26). In both studies, the test material was administered for 24 hr under occluded conditions to groups of one (low dose) or two (high dose) male/female rabbits. During the 14-day post-dosing observation period, symptoms of exposure included loss of appetite and inactivity. The viscera were not affected, and no deaths occurred. Although group sizes are less than optimal, the relatively high doses administered as well as the relatively low dermal bioavailability anticipated for dilute EP-306 components suggest that the available data adequately address the acute dermal hazard posed by EP-306.

### 5.3 Inhalation Toxicity

A four-hour LC<sub>50</sub> for adipic acid (99.8% purity) was measured in Sprague-Dawley rats (27). In this study, groups of 10 male and 10 female rats received nose-only exposures to adipic acid dust (MMAD 50% of 3.5 µm) at analytically measured concentrations of 5.4 mg/l and 7.7 mg/l for 4 hours and were observed for 14 days post-exposure. No clinical findings or mortalities were observed at either exposure concentration. As the recommended (OECD 403) limit-dose for respirable materials is 5 mg/l, this study of adipic acid is considered an adequate test for the acute inhalation toxicity of EP-306.

**Recommendation:** No additional acute toxicity studies are recommended. The available data fill the HPV required endpoints for acute toxicity. The weight of evidence shows that EP-306 exhibits low acute toxicity by all routes of exposure, particularly dermal exposure, the route most likely to be involved in human exposures under current use / manufacturing conditions.

## 6.0 Mammalian Genotoxicity

The SIDS/HPV requirement for genetic toxicity screening includes two end-points, one sensitive to point mutations and one sensitive to chromosomal aberrations. For EP-306, these requirements are fulfilled by multiple studies on either EP-306 itself or surrogate chemicals (Table 8).

### 6.1 Genotoxicity *in vitro*

The mutagenic potential of EP-306 was evaluated under GLP conditions in a *Salmonella typhimurium* (OECD 471) and *Escherichia coli* (OECD 472) reverse mutation assay using the standard plate and preincubation tests (28). Four strains of *S. typhimurium* (TA 98, TA 100, TA 1535, TA 1537) and *E. coli* WP2 uvrA were exposed with and without S-9 activation to five doses of EP-306 ranging from 40 µg/plate to 10,000 µg/plate. No mutagenic response was observed. In both the standard plate and preincubation tests, a bacteriotoxic effect was observed in some strains at doses  $\geq 5,000$  µg/plate. These results are supported by two standard plate incorporation assays with EP-306 surrogates. In the first, five strains of *S. typhimurium* (TA 98, TA 100, TA 1535, TA 1537, TA 1538) and *E. coli* WP2 uvrA were exposed with and without S-9 activation to five doses of adipic acid (purity unspecified) ranging from 33 µg/plate to 10,000 µg/plate (29). In the second, five strains of *S. typhimurium* (TA 98, TA 100, TA 1535, TA 1537, TA 1538) were exposed under GLP conditions with and without S-9 activation to five doses of C4 to C6 dicarboxylic acid mixture ranging from 30 µg/plate to 3,000 µg/plate (30). No mutagenic response was observed in either study. In a pretest, cytotoxicity was observed with the dicarboxylic acid mixture at a dose of 5,000 µg/plate.

**Table 8. Genetic Toxicity of EP-306 and Surrogate Chemicals**

Endpoint	EP-306	Adipic Acid	C4 to C6 Dicarboxylic Acid Mixture
Mutagenic?	No Ames Assay (28)	No Ames Assay (29)	No Ames Assay (30)*
Clastogenic?	No Data	No <i>In vivo</i> Cytogenetic Assay (31) Dominant Lethal Assay (32)	No <i>In vivo</i> Cytogenetic Assay (33)**

\*Containing: glutaric acid (30-35%), succinic acid (9-11%), adipic acid (6-9%), nitric acid (0.5-3%), and water (42-54.5%)

\*\*Containing: glutaric acid (28.8%), succinic acid (12.7%), adipic acid (7.9%), nitric acid (1.8%), and water (48.8%)

### 6.2 Genotoxicity *in vivo*

The clastogenic potential of EP-306 was evaluated using results of studies performed with surrogate chemicals. In the acute portion of an *in vivo* cytogenetic assay in rats, male rats (15/group) received a single dose of adipic acid at one of four doses ranging from 3.75 mg/kg to 5000 mg/kg and were killed at 6 hours, 24 hours and 48 hours post-exposure (31). In the subacute portion of the study, male rats (5/group) were exposed once daily for five days to four doses of adipic acid ranging from 3.75 mg/kg to 2,500 mg/kg; rats were killed 6 hours after the last dose. Fifty metaphase bone marrow cells/animal were evaluated for chromosomal aberrations. Results of both procedures were negative, although a few aberrations within the historical control range were noted in the middle two doses of the subacute study. In a dominant lethal assay (32), rats were exposed to adipic acid using the same acute / subacute procedure and doses previously described. Males were then mated with two virgin females each week for either 8 (acute study) or 7 (subacute study) consecutive weeks. Females were killed and examined for evidence of implantations fourteen days after separation from the males. Although significant differences (decreased implantations, decreased corpora lutea, increased preimplantation loss) were occasionally observed between the control and experimental groups at the lower two doses, adipic acid was judged not to produce dominant lethal changes as the observed effects revealed no dose-response or time-trend response.

The results with adipic acid are supported by a GLP *in vivo* cytogenetic assay of a C4 to C6 carboxylic acid mixture in rats (33). Male (2750 mg/kg) and female (1375 mg/kg) rats received a single dose of a C4 to C6 carboxylic acid mixture and were killed 6 hours (8 males/5 females), 18 hours (5 of each sex) and 30 hours (5 of each sex) post-exposure. Fifty metaphase bone marrow cells/animal were evaluated for chromosomal aberrations. Results were negative despite achieving / exceeding of the maximum tolerated dose as evidenced by toxic signs in both sexes (e.g., inactivity, decreased body tone, piloerection, vocalization at touch) and the death of 3 of 18 males.

**Recommendation:** No additional testing is recommended as the SIDS requirements are met by the battery of *in vitro* and *in vivo* studies on EP-306 and surrogate chemicals that are sensitive to both mutagenic and clastogenic events. The data indicate EP-306 has a low genotoxic potential.

## 7.0 Long-Term Mammalian Toxicity

As indicated in the Introduction, EP-306 is a site limited intermediate with limited potential for exposure. As such, testing for repeated dose toxicity and reproductive toxicity are not required under the US HPV Challenge program (34). However, sufficient chronic toxicity data are available on adipic acid, the major component of EP-306 to meet the requirements for a non site limited material. These data can also be used as a surrogate for 6-hydroxycaproic acid, the second most prevalent component in EP-306, as 6-hydroxycaproic acid will be metabolically converted to adipic acid once absorbed into the body. As these two chemicals are the most prevalent components in EP-306, it is likely that they also constitute a major fraction of the unidentified esters in EP-306. In the body, the EP-306 esters would be hydrolyzed by carboxyesterases to their alcoholic and acidic components. Thus, the total fraction of these two chemicals in EP-306 is thought to comprise approximately 90% of all organic components in EP-306. On this basis, toxicity data on adipic acid can serve as an appropriate reference for the chronic hazard posed by EP-306. Data on adipic acid are presented below.

### 7.1 Repeated Dose and Reproductive Toxicity

In a two-year chronic feeding study (35), male and female rats were exposed to adipic acid in the diet at concentrations of: 0%, 0.1%, 1%, 3%, and 5% (males) and 0% and 1% (females). The average daily consumption of adipic acid over the study period was: 16.9 mg, 175 mg, 506 mg, and 814 mg per rat for the four dose groups. Assuming an average rat body weight of 0.35 kg, these consumption values correspond to adipic acid doses of 48 mg/kg-day, 500 mg/kg-day, 1450 mg/kg-day, and 2330 mg/kg-day. In males, the percent survival of each test group was greater than that of the control group. Body weights and food consumption in the two highest dose groups were significantly less than the control group. No differences were observed in tumor incidence, clinical signs, weights of ten unspecified organs, or in gross and microscopic histopathology of 15 unspecified organs. Clinical chemistry and hematology data were not reported. The same endpoints were evaluated in females, except that tissue weights were limited to four unspecified organs. No significant differences were observed between the experimental and control groups. These data indicate that the No Observed Adverse Effect Level (NOAEL) for adipic acid is approximately 500 mg/kg-day with 4-fold greater doses associated with effects on only food consumption and body weight gain.

There are no guideline studies on the reproductive toxicity of adipic acid. However, the SIDS Manual (36) indicates that the reproductive endpoint can be addressed when a 90-day repeated dose study demonstrates no effect on the reproductive organs and data from a developmental toxicity study are available. The study discussed above did not detect toxicity to the reproductive tissues evaluated: testes (weights and histopathology), ovaries (histopathology), and uterus (histopathology). This information, combined with the developmental toxicity data described below, indicate that adipic acid / EP-306 does not pose a significant reproductive hazard.

**Recommendation:** No additional testing is recommended, as the available data are sufficient to assess the repeated dose toxicity and reproductive organ pathology of EP-306.

## 7.2 Developmental Toxicity

Pregnant rats (24-25 per group) were exposed to adipic acid by gavage on days 6-15 of gestation at one of four doses ranging from 2.9 mg/kg to 288 mg/kg (38). On day 20, the dams were killed and the number of implantation and resorption sites as well as live and dead fetuses were recorded. All fetuses were examined for external congenital abnormalities; live fetuses were weighed. Visceral and skeletal examinations of the fetuses were also performed. No effects were observed on: maternal or fetal survival, reproductive outcomes, embryotoxicity, or teratogenicity. The maternal and developmental NOAELs in the rat were determined to be > 288 mg/kg. In another developmental toxicity study (39), pregnant rabbits (13-20 per group) were exposed to adipic acid by gavage on days 6-18 of gestation at one of four doses ranging from 2.5 mg/kg to 250 mg/kg. The maternal and fetal endpoints examined were comparable to those discussed above in the rat. No effects were observed on: maternal or fetal survival, reproductive outcomes, embryotoxicity, or teratogenicity. The maternal and developmental NOAELs in the rabbit were determined to be > 250 mg/kg. Although maternal toxicity was not demonstrated in these developmental toxicity studies, it should be taken into consideration that adipic acid is regulated by the FDA as GRAS (generally recognized as safe) and is a permitted direct food additive (at levels as high as 1.3% for some food items) considered suitable for all segments of the population, including pregnant females (39).

**Recommendation:** No additional developmental toxicity testing is required as the available data are sufficient to assess the developmental hazard posed by EP-306.

## 8.0 Conclusions

It is concluded that the available information on EP-306 fills the data requirements for all endpoints specified in the EPA HPV Challenge program: physical-chemical parameters, environmental fate, aquatic toxicity and mammalian toxicity. Although the available studies do not meet all the requirements of the current OECD guidelines, taken together the information provided a reliable hazard assessment, particularly given the use of EP-306 as a site-limited intermediate. On this basis, further testing is not recommended.

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## 10.0 ROBUST SUMMARIES

[SEE IUCLID DATA SET: EP-306]